Prevalence and characteristics of meticillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009

Inaugural-Dissertation
zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

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genehmigt auf Antrag von

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Prof. Dr. Brigitte Berger-Bächli, Korreferentin

Zürich 2010
In großer Dankbarkeit und Liebe meinen Eltern Ulrike und Erich und Vinzenz
Beitrag von Helen Huber (Teil A) zum Thema:

Prevalence and characteristics of meticillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009

- Untersuchung von 2'662 Proben von 148 Schweinehaltern, 133 Tierärzten, 179 Schlachthof-Mitarbeitern, 800 Schweinen, 300 Kälbern, 400 Rindern, 100 Geflügelherden sowie von Lebensmitteln tierischer Herkunft (n=460) mittels zweistufiger Anreicherung und Umzüchtung auf MRSA-Selektivmedium auf das Vorkommen von Meticillin-resistenten *Staphylococcus aureus* (MRSA).
- Untersuchung von 142 aus bovinen Mastitismilch-Proben isolierten *S. aureus*-Stämmen mittels MRSA-Selektivmedium auf Meticillin-Resistenz.
- Identifizierung der MRSA mittels *S.aureus*-spezifischer PCR und anschliessender PCR zur Überprüfung auf das Vorhandensein des *mecA*-Gens.
- Antibiotika-Resistenz-Prüfung der MRSA-Stämme (Plättchendiffusions-Test, E-Test).
- Pulsfeld-Gelelektrophorese der MRSA-Stämme nach DNA-Verdau mit dem Restriktionsenzymes *EagI*.
- Untersuchung der MRSA-Stämme auf das Vorkommen von Panton-Valentine Leukocidin und den Staphylokokken-Enterotoxine A bis D mittels PCR.
- Erstellung des Manuskriptes zur Publikation in Eurosurveillance und der Dissertationsschrift.

10. Juni 2010
A total of 2,662 samples, collected from March to September 2009 in Switzerland, were tested for the presence of meticillin-resistant *Staphylococcus aureus* (MRSA). The collection comprised nasal swabs from 148 pig farmers, 133 veterinarians, 179 slaughterhouse employees, 800 pigs, 300 calves, 400 cattle, 100 pooled neck skin swabs from chicken carcasses, and 460 food samples of animal origin. Moreover, 142 *S. aureus* strains, isolated from bovine mastitis milk, were included in the study. Twenty samples (c 1%; four veterinarians, 10 pigs, three calves, one young bull, and two mastitis milk samples) tested positive for MRSA. Genotyping of the MRSA strains was performed by multilocus sequence typing, *spa*- and SCC*mec*-typing, and revealed ST398 (n=18), ST18 (n=1), ST1 (n=1), *spa* types t011 (n=7), t034 (n=11), t064 (n=1), t127 (n=1), and SCC*mec* types IV (n=4) and V (n=16). The 20 MRSA strains were subjected to antibiotic susceptibility testing and pulsed-field gel electrophoresis using the restriction enzyme *EagI*. Supplementary PCR reactions were performed to investigate the presence of Panton-Valentine leukocidin and staphylococcal enterotoxins A to D.

**Introduction**

Meticillin-resistant *Staphylococcus aureus* (MRSA) has become a pathogen of increasing importance in hospitals, the community, and in recent years also in livestock. MRSA associated with livestock (LA-MRSA) have been reported worldwide in many species, but mainly in pigs, with sequence type (ST) 398 found most frequently [1-3]. With regard to humans in contact with farm animals, Voss *et al.* described in 2005 that Dutch pig farmers at a 760-fold risk of being colonised with MRSA compared to the general Dutch population [4]. In an international study, Wulf *et al.* found MRSA in 12.5% of veterinarians originating from all over the world [5]. These studies strongly suggest that people working with livestock are at a potential risk of becoming MRSA carriers and hence are at an increased risk of infections caused by MRSA. To date, there is no comprehensive data on the situation of LA-MRSA in Switzerland. The aim of this study was to evaluate the occurrence of MRSA in people in contact with livestock, in farm animals, and in food of animal origin, and to investigate genotypic characteristics as well as phenotypic resistance data of isolated strains.

**Methods**

From March to September 2009, we collected and analysed a total of 2,662 samples from humans, livestock, and food of animal origin. In terms of humans with contact to farm animals, we analysed nasal swabs from 148 pig farmers attending meetings on swine breeding, 133 veterinarians participating in a course on castration of piglets, and 179 slaughterhouse employees working in two different abattoirs. Livestock was sampled at slaughter: Nasal swabs from pigs (n=800), calves (n=300), and cattle (n=400) were collected, as well as neck skin samples (n=100) from chicken carcasses, pooled by flock. Sampled animals originated from more than 830 farms distributed throughout Switzerland. In terms of food, 100 samples of bulk tank milk (BTM), 200 samples of raw-milk cheese, and 160 minced pork and beef samples were tested. Furthermore, 142 *S. aureus* strains from clinical cases of bovine mastitis were integrated in the study.

After a two-step enrichment procedure in Mueller-Hinton broth supplemented with 6.5% NaCl (24 h at 37°C) and in phenol red mannitol broth supplemented with 75 mg/L aztreonam and 5 mg/L cefoxitin (24 h at 37°C), the samples were plated onto Oxoid Brilliance MRSA Agar (Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37°C. In addition, 142 *S. aureus* strains isolated from bovine mastitis milk were directly streaked onto Oxoid Brilliance MRSA Agar. Presumptive positive colonies were confirmed as *S. aureus* by species-specific 23S rDNA PCR [6] and as MRSA by PCR detection of mecA gene [7], before further characterisation.
by multilocus sequence typing (MLST) [8], spa typing [9], and determination of staphylococcal cassette chromosome mec (SCCmec) type [10] was performed. Moreover, strains were tested by PCR for lukS-PV and lukF-PV [11] encoding Panton-Valentine leukocidin (PVL), and for sea to sed [12] encoding staphylococcal enterotoxins (SE) A to D. To demonstrate phenotypic properties, strains were tested for their antibiotic resistance patterns using the disk diffusion method (BD BBL Sensi-Disc; Becton, Dickinson and Company, Sparks, MD, US). The following disks were used: ampicillin, cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, penicillin, rifampicin, sulphamethoxazole/trimethoprim, tetracycline, and vancomycin. Susceptibility testing with cefoxitin is recommended by Witte et al., especially for low-level resistant MRSA [13]. Etest (AB Biodisk, Solna, Sweden) was additionally used for cefoxitin and oxacillin resistance testing. The results of antibiotic susceptibility testing were interpreted according to the guidelines of

**Table**

Characterisation of meticillin-resistant *Staphylococcus aureus* strains, Switzerland, March-September 2009 (n=20)

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number of isolates</th>
<th>Sequence type (ST)</th>
<th>spa type</th>
<th>SCCmec type</th>
<th>PVL</th>
<th>SE</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig NS</td>
<td>8</td>
<td>398</td>
<td>t034</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>Amp, Cef, Cli, Ery, Oxa, Pen, Tet</td>
</tr>
<tr>
<td>Pig NS</td>
<td>1</td>
<td>398</td>
<td>t034</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>Amp, Cef, Cli, Ery, Gen, Oxa, Pen, SxT, Tet</td>
</tr>
<tr>
<td>Pig NS</td>
<td>1</td>
<td>398</td>
<td>t034</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>Amp, Cef, Oxa, Pen</td>
</tr>
<tr>
<td>Calf NS</td>
<td>3</td>
<td>398</td>
<td>t011</td>
<td>V</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Cattle NS</td>
<td>1</td>
<td>1</td>
<td>t127</td>
<td>IV</td>
<td>-</td>
<td>-</td>
<td>Amp, Cef, Ery, Oxa, Pen, Tet</td>
</tr>
<tr>
<td>Vet NS</td>
<td>1</td>
<td>398</td>
<td>t034</td>
<td>V</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Vet NS</td>
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<td>Mastitis milk</td>
<td>2</td>
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<td>V</td>
<td>-</td>
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<td>Amp, Cef, Cli, Ery, Oxa, Pen, Tet</td>
</tr>
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</table>

the Clinical and Laboratory Standards Institute (CLSI). Furthermore, macrorestriction profiling with pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Eagl* was performed [2].

**Results**

A total of 20 MRSA strains were detected (Table). They derived from samples from four (3.0%) of 133 veterinarians, 10 (1.3%) of 800 pigs, three (1.0%) of 300 calves, one (0.3%) of 400 cattle, and from two (1.4%) of 142 mastitis milk samples (Figure). In contrast, MRSA were not found in pig farmers, slaughterhouse employees, poultry, and in food samples such as BTM, raw milk cheese, and minced meat.

The four strains isolated from veterinarians belonged to ST8 and ST398 (Table). The strain of ST8 harbored *spa* type t064, SCC*mec* type IV, was negative for PVL, and positive for SEA and SEB. This strain of ST8 from a veterinarian was thus the only one harbouring genes encoding staphylococcal enterotoxins. The three strains belonging to ST398 tested negative for PVL and SE. One was *spa* type t034 and SCC*mec* type V. The other two strains belonged to *spa* type t011 and SCC*mec* type IV. The 10 MRSA strains isolated from pigs originated from eight different farms in seven regions of Switzerland, and all belonged to ST398, *spa* type t034, SCC*mec* type V and tested negative for PVL and SE. These characteristics are the same as those found in one strain from a veterinarian. The results obtained from strains of calves were similar, with the only difference that all three strains were grouped into *spa* type t011. Two strains from veterinarians were also of ST398 and *spa* type t011 but belonged to SCC*mec* type IV. The strain found in a young bull showed different characteristics. It belonged to ST1, *spa* type t128, SCC*mec* type IV, and was negative for SE and PVL. The two strains isolated from mastitis milk both belonged to ST398, *spa* type t011, SCC*mec* type V and were negative for PVL and SE. According to these typing results, the strains isolated from mastitis are identical to the ones isolated from calves.

Digestion with *Eagl* as restriction enzyme provided uniform band patterns for nine of 10 strains isolated from pigs. The three strains from calves and the two strains from mastitis milk showed uniform patterns as well and were related to the ones from pigs. The three MRSA strains of type ST398 isolated from veterinarians showed different patterns.

All 20 MRSA strains were susceptible to vancomycin and rifampin. All but two strains were susceptible to gentamicin and sulphmethoxazole/trimethoprim and all but one were susceptible to ciprofloxacin (Table). Of the 16 strains isolated from animals (livestock and mastitis milk), all were resistant to four beta-lactams (ampicillin, cefoxitin, oxacillin, penicillin), 15 were resistant to erythromycin and tetracycline, and 14 to clindamycin. Of the four MRSA strains isolated from veterinarians, two strains were phenotypically susceptible to oxacillin but resistant to cefoxitin, with the disk diffusion as well as the Etest method, and therefore were low-level resistant MRSA.

**Discussion**

Our results show that MRSA, and ST398 in particular, are present in Swiss livestock but still occur in low numbers. Compared to the herd level prevalence of 81% in Dutch pigs [1], the herd level prevalence of MRSA ST398 of 2.9% in pigs and 1.6% in calves found in our samples was low. In view of the small proportion of MRSA-positive animals found in Swiss livestock, the related risk of food contamination and transmission of MRSA to people in contact with livestock does currently not seem of particular importance in Switzerland. In our study, MRSA prevalence in veterinarians was 3%. This finding is favourable compared to results published by Wulf *et al.* [5], who found 12.5% of veterinarians attending an international congress on pig health to be MRSA carriers. Contrary to what was recently reported by De Boer *et al.* [14], we found no MRSA in meat samples. Our findings in raw-milk cheese and BTM are in good accordance with recently published data from the United States [15]. The fact that we detected no MRSA in poultry, pig farmers, slaughterhouse employees, and food samples is especially noteworthy since these results are different to findings published for other countries [3,4,13,16].

Possible explanations for the low MRSA prevalence in Switzerland may be the restrictive and controlled use of antibiotics in farming, a good health status of pig herds compared to many countries in the European Union, and the fact that the importation rate of live pigs in Switzerland is very low (<1%) [17].

The two non-ST398 strains with ST1 (young bull) and ST8 (veterinarian) found in our study are of sequence types usually considered as community-associated MRSA. Juhász-Kasanyitzky *et al.* reported MRSA of ST1, *spa* type t127, SCC*mec* type IV in humans and bovines [18]. Moreover, such MRSA were also isolated from horses and horse personnel in Austria [19]. The presence of MRSA ST8, *spa* type t064, SCC*mec* type IV was recently reported in horses by Weese and van Duijkeren [20].

All LA-MRSA of ST398 found in our study, belonged to two *spa* types (t034, t011), which represent the most common *spa* types in European LA-MRSA. Among our samples, *spa* type t011 was associated with bovine strains, whereas *spa* type t034 was associated with strains isolated from pigs. The two MRSA strains we isolated from mastitis milk were of ST398 and *spa* type t011, which is comparable to the recent results of Vanderhaeghen *et al.* [21]. It seems quite understandable that veterinarians can carry both, *spa* type t011 and t034, since veterinarians in Switzerland usually visit pig and cattle facilities. Visiting many different farms per day can also be an explanation for the higher
percentage of MRSA carriers among veterinarians compared to pig farmers.

Conclusion

MRSA, and especially LA-MRSA ST398, have entered Swiss farming operations but to date occur in low numbers. This low prevalence suggests that at the moment there is only a limited risk of MRSA transmission from livestock to humans and to food of animal origin. To maintain this situation, further efforts within the field of veterinary public health are of major importance and it is necessary to establish a monitoring system for further trend analysis.

Acknowledgements

We thank the staff of the slaughterhouses involved in this study for facilitating access to their operation and for assistance with the collection of data. Xavier Sidler for his great effort in making the sampling of veterinarians and pig farmers possible, and Herbert Hächler for his precious assistance in performing antibiotic susceptibility testing.

References

Acknowledgements

An dieser Stelle bedanke ich mich ganz herzlich bei allen, die zur Entstehung dieser Arbeit beigetragen haben.

Besonderer Dank geht an:

Herrn Prof. Roger Stephan, Institut für Lebensmittelsicherheit und -hygiene, Vetsuisse Fakultät Universität Zürich, für die Möglichkeit zur Erstellung dieser Dissertation, die fachliche Beratung und wertvolle Unterstützung in allen Belangen sowie die Übernahme des Referates.

Frau Prof. Dr. Brigitte Berger-Bächli, Institut für Medizinische Mikrobiologie der Universität Zürich, für die Übernahme des Korreferates.

Herrn PD Dr. Claudio Zweifel, Institut für Lebensmittelsicherheit und -hygiene, Vetsuisse-Fakultät Universität Zürich, für seinen immensen Einsatz sowie die grosszügige und unbezahlbare Hilfe bei unzähligen Fragen aller Art.

Frau Nicole Giezendanner für ihren grossartigen Einsatz bei der Laborarbeit und ihre Geduld.

Herrn Dr. Xaver Sidler für seine grosszügige Hilfe und die Ermöglichung der Probenentnahme bei Tierärzten und Schweine-Haltern.

Herrn PD Dr. Herbert Hächler für seine Hilfe bei der Antibiotika-Resistenzprüfung.

Frau Corinna Seibert für die zuverlässige Herstellung der Medien und Nährösungen.

Dem ganzen ILS-Team für die gegenseitige Hilfe bei Laborarbeiten.

Frau Dr. Margrit Stamm und den Mitarbeitern der Bell Poulet AG in Zell für die Zusammenarbeit.

Herrn Dr. Martin Bürkli und den Mitarbeitern der Frischfleisch AG Sursee für die Zusammenarbeit.

Frau Simona Blatter und Frau Evelyne Michel für unzählige Lacher und gute Gespräche.
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26. April 2010